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कृषि एवं खाद्य पदार्थों में लिन्डेन (गामा बीएचसी) के अवशेष ज्ञात करने की पद्धति

Indian Standard

DETERMINATION OF LINDANE (GAMMA-BHC) RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES

ICS 67.040 ; 71.040.40

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

November 1995

Price Group 2

Pesticides Residue Analysis Sectional Committee, FAD 34

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Pesticides Residue Analysis Sectional Committee had been approved by the Food and Agriculture Division Council.

Lindane (Gamma-BHC) formulations are extensively used in agriculture for the control of insect pests. This standard will enable the food and health authorities and others engaged in the field to follow a uniform test procedure for the estimation of lindane (Gamma-BHC) residues in various agricultural and food commodities.

In the preparation of this standard, due consideration has been given to the limits of lindane (Gamma-BHC) residues laid down under the provisions of *Prevention of Food Adulteration Act,* 1954 and *Rules* framed thereunder. The test method is sensitive to the maximum level of residues prescribed under these *Rules.*

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values *(revised)*'.

Indian Standard

DETERMINATION OF LINDANE (GAMMA-BHC) RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES

1 SCOPE

This standard prescribes the gas chromatographic method for determination of lindane (Gamma-BHC) residues [1, 2, 3, 4, 5, 6-hexachlorocyclohexane (mixed isomers)] in agricultural and food commodities.

NOTE — This method has a detection limit of 0.01 μg/g (0.01 ppm).

2 REFERENCE

IS 11380 (Part 1) : 1985 'Method of sampling for the determination of pesticide residues : Part 1 Agricultural and food commodities', is a necessary adjunct to this standard.

3 SAMPLING

The representative samples for the purpose of estimating lindane residues in the agricultural and food commodities shall be drawn in accordance with IS 11380 (Part 1) : 1985.

4 SAMPLE STORAGE

Store the sample as such when chances of its degradation do not exist and the quantity is small. Otherwise carry out the extraction and store the extract depending upon the nature of the sample, keep the sample or its extract either in deep-freezer at −15°C or in refrigerator until taken up for analysis. Ensure that the samples do not absorb or loose moisture during storage. Avoid undue long storage periods.

5 APPARATUS

5.1 Gas Chromatograph

The gas chromatograph shall be fitted with an electron capture detector. The following operating parameters are suggested, which can be changed provided standardization is done:

Column : Glass, 184 cm length × 2 mm I.D. ; packed with 1.5 percent SP-2250 and 1.95 percent SP-2401 on Supelcoport (100-120 mesh) or 1.5 percent OV-17 and 1.95 percent QF-1 on Gas Chrom Q Chromosorb W-HP (80-120 mesh).

Temperature:

Column Oven	190°C
Injection Port	210°C
Detector	220°C

Carrier gas and flow rate: Nitrogen; 60 ml/minute

5.2 Microlitre Syringe—10 μl capacity

5.3 Waring Blender or Equivalent

5.4 Centrifuge Machine along with Centrifuge Tubes

5.5 Clean-Up Apparatus

5.6 Glass Column — 50 cm length and 1.4 cm ID.

5.7 Soxhlet Apparatus

5.8 Vacuum Flask

5.9 Blender Jar

5.10 Separatory Funnel

5.11 Round Bottom Flask

5.12 Rotary Vacuum Evaporator

5.13 Glass Funnel

5.14 Beaker

6 REAGENTS

6.1 Acetone, glass re-distilled.

6.2 *n*-Hexane, glass re-distilled.

6.3 Sulphuric Acid, concentrated.

6.4 Acetonitrile, glass re-distilled.

6.5 Celite, 545 or equivalent.

6.6 Sodium Chloride Solution — 2 and 10 percent (*m/v*) aqueous solution.

6.7 Sodium Chloride Solution, saturated.

6.8 Sodium Sulphate, anhydrous.

6.9 Sodium Sulphate

6.10 Distilled Water

6.11 Litmus Paper

6.12 Whatman No. 2 Filter Paper

6.13 Activated Silica Gel — column chromatography grade activated at 110°C for 6 hours.

6.14 Benzene, glass re-distilled.

6.15 Petroleum Ether, glass re-distilled.

6.16 Reference Standard Lindane, of known purity.

7 EXTRACTION

7.1 Weigh 50 g homogenized sample into a waring blender, add 50 g anhydrous sodium sulphate, 150 ml acetone and blend for 2-3 minutes at moderate to high speed. Filter the blended material through 5 g celite or equivalent under vacuum. Remove suction as soon as liquid is filtered. Transfer the residue to the blender jar and extract with an additional 100 ml acetone as previously described. Filter as before, combine the filtrates. Measure the volume of the filtrate and transfer one-half of it (equivalent to 25 g of sample) to a 1 000-ml separatory funnel. Add 100 ml *n*-hexane and shake vigorously for one minute, venting pressure. Add 2 percent sodium chloride solution, equal to four volumes of acetone extract. Mix thoroughly by vigorous tumbling action for 15 seconds, vent and allow the two layers to separate.

7.1.1 Collect the lower aqueous and upper *n*-hexane layers separately. Transfer the aqueous phase to a separatory funnel, add 50 ml *n*-hexane and shake, as previously described. Separate the two phases and extract the aqueous phase with another portion of 50 ml *n*-hexane. Combine the extracts and wash twice with 100 ml of distilled water. If emulsion fumes, wash with 2 percent sodium chloride solution or add 5 ml saturated sodium chloride solution to the separatory funnel. Discard the washings and draw off *n*-hexane layer into a round-bottom flask through Whatman No. 2 filter paper containing 5 g anhydrous sodium sulphate. Evaporate the filtrate to about 10 ml in an evaporative concentrator below 40°C temperature.

7.2 Cereals, Pulses and Spices

7.2.1 Place 20 g of ground sample in a thimble of Soxhlet apparatus. Extract with 150 ml acetonitrile for 8 hours using a 500-ml round-bottom flask.

7.2.2 Transfer the extract to an 1 000-ml separatory funnel. Add to it three volumes of 10 percent aqueous sodium chloride solution and 2 percent 100 ml *n*-hexane. Shake it for few seconds and vent to relieve any pressure developed. Continue shaking for about one minute. Vent again and allow the two layers to separate. Remove the aqueous and *n*-hexane layer separately. Transfer the aqueous again to the separatory funnel, add 50 ml *n*-hexane and shake as previously outlined. Separate the two phases and mix the *n*-hexane layer with the first hexane fraction. Re-extract the aqueous phase again with 50 ml *n*-hexane and add the hexane fraction with the previously obtained hexane fractions. Evaporate the combined *n*-hexane extract to about 10 ml in a rotary vacuum evaporator at temperature below 40°C.

7.3 Oilseeds and Nuts

7.3.1 Transfer 20 g ground sample to the thimble of a Soxhlet apparatus. Add 150 ml acetonitrile and extract for 8 hours.

7.3.2 Transfer the extract to a 1 000 ml separatory funnel, add three volumes of 10 percent aqueous sodium chloride solution, 100 ml *n*-hexane and shake well. Drain the *n*-hexane layer with a 500 ml separatory funnel. Add 100 ml *n*-hexane to aqueous layer in the previous separatory funnel, shake well and subsequently drain the *n*-hexane layer to the 500 ml separatory funnel. Wash the combined hexane fractions twice with 100 ml of distilled water taking care to minimise emulsion formation. Discard the washings. Filter the hexane layer into a 500 ml round-bottom flask through a filter containing 10 g of sodium sulphate. Rinse the separatory funnel with *n*-hexane and concentrate the extract to about 50 ml in a evaporative concentrator.

7.4 Milk

7.4.1 Weigh 50 g representative sample in a beaker and transfer to a waring blender, rinsing the beaker with 5 ml distilled water thrice. Add 50 ml acetone, 150 ml *n*-hexane and blend for 2 minutes at moderate speed. Transfer to centrifuge tubes, rinsing with 20 ml *n*-hexane and then with 10 ml distilled water and adding rinsing to the centrifuge tubes. Centrifuge at approximately 2 000 rotations per minute for 10 minutes.

7.4.2 Draw off *n*-hexane layer and transfer it to a 500 ml round bottom flask after passing through a 1-cm layer of anhydrous sodium sulphate.

7.4.3 Add 50 ml *n*-hexane to the centrifuge tube, shake and then centrifuge again. Draw off the upper *n*-hexane layer, combine with the earlier *n*-hexane extract (*see* 7.4.2) and evaporate the combined extract using a rotary vacuum evaporator to about 10 ml.

7.5 Butter and *Ghee*

7.5.1 Dissolve exactly 3 g fat in 100 ml petroleum ether or *n*-hexane and transfer to the glass column packed with activated silica gel. Add 40 ml concentrated sulphuric acid to the 250-ml separatory funnel placed on the top of the column and let it drip at 4 ml per minute on to the column. The lower layer containing the acid and fat is continuously discarded.

7.5.2 Dissolve another 2 g fat in 10 ml petroleum ether or *n*-hexane and transfer to the extraction column. Add again 40 ml sulphuric acid drop-wise at the rate of 4 ml per minute and discard the lower layer. Repeat the treatment using 10 ml sulphuric acid each time till the lower layer is clear. Transfer the upper pertoleum ether or *n*-hexane layer through a glass funnel plugged with glass wool into a 250 ml separatory funnel and wash with distilled water till neutral to litmus. Concentrate the petroleum ether or *n*-hexane extract to the suitable volume and analyse.

8 CLEAN-UP

8.1 Set up the clean-up apparatus. Fill the glass column with 10 g activated silica gel and top with 2 cm of anhydrous sodium sulphate. Wash the column with 100 ml *n*-hexane and discard the eluate. Transfer the extract (*see* **7.1.1**, **7.2.2**, **7.3.2**, **7.4.3**, and **7.5.2**) to glass column. Rinse the container of the extract with 10 ml portions of *n*-hexane and transfer the rinsings also to the column. Add 40 ml concentrated sulphuric acid to the 250 ml separatory funnel placed on the top of the column and let it drip at 4 ml per minute onto the centre of the column. Discard the lower layer containing acid and digested fat. Repeat the treatment using 10 ml concentrated sulphuric acid each time till the lower layer becomes completely clear. Transfer the *n*-hexane layer through a glass funnel plugged with glass wool, into another 250 ml separatory funnel and wash with 20 ml portions of distilled water till the washing becomes neutral to litmus. Concentrate the *n*-hexane fraction to suitable volume and analyse.

9 ESTIMATION

9.1 Inject simultaneously a suitable aliquot of reference standard lindane and sample extract into the column using a microlitre syringe. Identify the peak by the retention times and measure the peak heights/areas.

10 CALCULATION

Lindane (gamma-BHC) residue, μ g/g

$$= \frac{A_1 \times V_2 \times V_3 \times C}{A_2 \times V_1 \times M} \times f$$

where

A_1 = peak height/area of the sample;

V_2 = volume in μl of standard lindane solution injected;

V_3 = total volume in ml of sample solution;

C = concentration in μg/g of the standard lindane solution;

f = recovery factor $= \dfrac{100}{\text{percent mean recovery}}$

A_2 = peak height/area of the standard lindane

V_1 = volume in μl of sample solution injected; and

M = mass in g of sample taken for analysis.

NOTE — Percent mean recovery is determined by taking untreated control sample to which known amount of lindane standard is added and analysed as described above.

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